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Biochemical, morphological, and yield responses of lady's finger plants to varying ratios of palm oil mill waste (decanter cake) application as a bio-fertilizer

Asha Embrandiri^{1*}, Rajeev Pratap Singh² and Mahamad Hakimi Ibrahim¹

Abstract

Background: Decanter cake is produced in large amounts, and its disposal is a major concern in palm-producing countries. Growth morphology and biochemical responses of lady's finger (*Abelmoschus esculentus*) plants grown on soil amended with different ratios of decanter cake (0%, 10%, 20%, and 30%) were investigated.

Results: The soil pH decreased (unamended soil), whereas the electrical conductivity increased as compared with the control. There was a significant difference in ascorbic acid content with the increase in treatment ratio. Phenol content was however highest in 20% of the amendment ($13.197 \pm 0.36 \text{ mg g}^{-1}$).

Conclusions: The results indicate that decanter cake amendments of up to 10% may be a probable substitute for inorganic fertilizers with respect to lady's finger (*A. esculentus*) plants due to high nutrient content, yield and biomass, as well as morphological characteristics. However, there were observable negative effects after 10% decanter cake amendment ratios.

Keywords: Decanter cake, Amendment ratios, Phenol content, Specific leaf area, Chlorophyll, Lady's finger

Introduction

The management of palm oil mill wastes has become a serious concern for oil-producing countries today because, if it is not dealt with properly, it could result in serious hazards to the environment (Singh and Agrawal 2010a, b, c; Singh et al. 2011a, b; Embrandiri et al. 2012). The processing of palm oil results in the production of huge quantities of residues also referred as by-products, namely palm oil mill effluent, palm kernel cake, decanter cake, empty fruit bunches (EFB), and palm kernel shell (Singh and Agrawal 2010a, b, c; Singh et al. 2011a, b). There are many studies going on using these by-products in various industries such as bio-fuels, lumber for wood, feed for animals, shells as activated carbon for water purification etc. However, it has also been suggested as a good bio-fertilizer, potting material and soil conditioning agent from several researchers

(Singh and Agrawal 2010a, b, c; Singh et al. 2011a, b; Embrandiri et al. 2012).

Unfortunately, the volume of waste produced supersedes its reuse, and most of it is still left unattended. This is mostly due to insufficient knowledge on the beneficial properties of these wastes. Land filling and incineration were the foremost cheapest options put forward by scientists to reduce the large volumes of solid wastes (Singh and Agrawal 2008). Recently, composting and vermicomposting have been gaining grounds as a better management option for these wastes because of their better NPK value (Singh et al. 2011a, b). These two processes add value and also cut down the volume of waste, making their land application easier (Yusri et al. 1995; Singh et al. 2011a, b). Majority of the studies indicated that crop production has benefitted from land application of organic residues due to the possibility of recycling of organic matter, N, P, and other plant nutrients (AdeOluwa and Adeoye 2008; Silva et al. 2010; Singh and Agrawal 2007, 2009, 2010a, b, c). According to Aisueni and Omoti (1999), the

* Correspondence: ashanty66@gmail.com

¹Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia, Penang 11800, Malaysia

Full list of author information is available at the end of the article

oil palm industry is one of the best sources of agricultural wastes that can be converted to organic fertilizers. The waste composition should however be determined prior to its land application as plants vary in their nutrient requirements. Kelepsei and Tzortzakis (2009) carried out studies using olive mill waste (OW), olive stone, and pulp in 10%, 30%, and 50% (v/v) ratios of olive mill wastes as a growth medium of lettuce and chicory by replacing part of the peat. Olive mill waste extract caused an increase in radical length of germinated seeds at 10^{-1} to 10^{-5} concentrations. Leaf lengths were reduced at the soil/OW (70:30) amendment as compared with the control. Leaf number and root length were unaffected by the treatments.

Wood and Lim (1989) recommended that rubber factory effluent (RFE) is favorable for oil palm production and some annual crops such as rice, pepper, vegetables, and tropical fruits. Correct application of RFE increased the oil palm yield up to 20% and rubber by 5% to 10%. The effects of using EFB composts spiked with cow dung for raising oil palm in the nursery was evaluated by AdeOluwa and Adeoye (2008). EFB/cow dung ratios (100:0, 90:10, 80:20, 70:30, and 60:40) as compost, cow dung, and mineral fertilizer (N/P/K/Mg, 12:12:17:2) were used from 0 to 13 months and added at a rate of 4.8 g N plant⁻¹ in the soil. They reported that composting of EFB in combination with cow dung led to a better performance of oil palm seedlings.

The present study was an attempt to identify the appropriate amendment ratio for decanter cake (DC) as a fertilizer supplement on *Abelmoschus esculentus* (lady's finger) plants to exploit the beneficial effect of nutrient enrichment and also to circumvent health risks due to nutrient accumulation after the application at different ratios. Morphological, biochemical, and yield response of lady's finger (*A. esculentus*) grown at different DC amendment ratios were assessed to comprehend the reaction of the test plant to a contradictory situation of beneficial effects of nutrient availability and harmful effects of excess nutrient accumulation.

Methods

Study site

The study was conducted at the nursery of the School of Industrial Technology, USM, Pulau Pinang from November 2010 to February 2011. The average temperature ranged between 30°C and 31°C in the nursery. The average annual rainfall was 2,670 mm, and relative humidity was 70% to 90%.

Sample collection

Decanter cake obtained from Malpom Industries Sdn Bhd, Nibong Tebal, Pulau Pinang, Malaysia was dried, powdered, and passed through a 2-mm sieve. Similarly, soil that was dug up from about 10 cm deep and was

taken from a nearby land was dried, homogenized, and passed through a 2-mm sieve.

Experimental design

The experiment consisted of planter boxes in a completely randomized design of about 25 cm in diameter and 15 cm in depth. Treatments in the ratios of 10%, 20%, and 30% (w/w) DC with unamended soil (0%) as control were thoroughly mixed and filled into the planter pots. Identical water regime was maintained and then left for 15 to 20 days for stabilization and mineralization of the organic matter. After the stabilization period was over, soil was collected in triplicates for physicochemical analysis. Prior to sowing, seeds of *A. esculentus* were soaked in distilled water for 3 min. About seven to eight seeds per treatment were sown manually at equidistant positions. After germination, the plants were thinned to three per pot, and identical temperature and humidity conditions were provided throughout the growth period.

Measurement of data

Physicochemical parameters

Soil samples of each treatment were collected in triplicates, crushed, air-dried, and sieved with a 2-mm mesh size. Decanter cake samples were also taken for further physicochemical analysis. The pH of samples was measured using a pH meter (Hach, sensION3, Loveland, CO, USA) in a 1:10 (w/v) suspension, while the electrical conductivity (EC) in 1:2 (w/v) was measured using the Hach sensION5 conductivity meter. A CHN analyzer (2010 PerkinElmer Instruments, Branford, CT, USA) was used to determine the C/N ratio of the amended soils. Bulk density test was carried out by taking 10 g of soil sample that was oven-dried at 105°C, slowly poured into a measuring cylinder, and gently tapped for compaction (expressed in g cm⁻³) (Radojevic and Bashkin 2006).

Specific gravity test was carried out, according to Radojevic and Bashkin (2006), by taking 10 g of dry sample in an empty glass bottle; the weight was taken thereafter. The bottle was then rinsed and filled to the brim with water, and the weight was taken. The formula used to calculate the specific gravity for the soil samples is as follows:

$$\text{Specific gravity} = \frac{M2 - M1}{M3 - M1},$$

where $M1$ = weight of empty glass bottle (g), $M2$ = weight of bottle and soil (g), and $M3$ = weight of bottle and water (g).

For analysis of metals in the soil and decanter cake samples, wet digestion method was followed: 1 g of air-dried soil sample was digested in 20 ml of triacid mixture ($\text{HNO}_3\text{:H}_2\text{SO}_4\text{:HClO}_4$) for 8 h at 80°C till transparency. The digested solution was then made up to 50 ml with de-ionized water and filtered with a Whatman no. 1 filter

paper. The filtrate was analyzed for various metals using an atomic absorption spectrophotometer (PerkinElmer Analyst 200 AAS 2007).

Morphological and growth parameters

Plant samples with intact roots were randomly taken from each treatment 65 days after sowing (DAS) and washed with running distilled water to remove soil particles that adhered on the roots. Growth parameters such as shoot length, root length, leaf area, and number of leaves were measured (Singh and Agrawal 2007, 2009). The leaf area was measured by a portable laser leaf area meter (Leaf area meter CI 203, CID Bio-Science, Camas, WA, USA). For biomass determination, separate root, shoot, and leaf parts were placed in the oven at 80°C until a constant weight was reached. The plant parts were then weighed separately, and biomass accumulation was expressed as grams per plant. Total plant biomass was estimated as the total dry weight of plant parts. Yield is expressed as fresh weight of fruits per plant at the time of harvest. For growth indices, such as leaf area ratio (LAR), leaf weight ratio (LWR), specific leaf area (SLA), and root shoot ratio (RSR) formulae by Hunt (1982), were used as shown:

$$\text{LAR (cm}^2 \text{ g}^{-1}\text{)} = \frac{\text{Leaf area}}{\text{Total biomass}},$$

$$\text{LWR (g g}^{-1}\text{)} = \frac{\text{Leaf dry weight}}{\text{Total biomass}},$$

$$\text{RSR (g g}^{-1}\text{)} = \frac{\text{Root dry weight}}{\text{Shoot biomass}},$$

$$\text{SLA (cm}^2 \text{ g}^{-1}\text{)} = \frac{\text{Leaf area}}{\text{Leaf biomass}}.$$

Photosynthesis pigments (chlorophyll and carotenoid) in fully expanded fresh leaves sampled at 45 DAS was estimated using the Machlachlan and Zalik (1963) and Duxbury and Yentsch (1956) methods. Phenol content was determined in accordance with the method of Bray and Thorpe (1954), and thiol content was estimated using the method of Fahey et al. (1978). Ascorbic acid concentration in fresh leaves was determined using the method of Keller and Schwager (1977).

Data analyses

Data obtained from the experiments were subjected to a one-way ANOVA test using the SPSS 18 software (SPSS Inc., Chicago, IL, USA; SPSS, Richmond, VA, USA) to assess the significance of differences and biochemical, physiological, and growth parameters of DC in comparison with those of the control (unamended soil). Mean

values were separated using Tukey's test at 5% probability level.

Results and discussion

The physicochemical parameters of decanter cake obtained from Malpom Industries are given in Table 1. With increasing decanter cake amendment ratios in the soil, there was a reduction in pH; however, EC increased significantly (Table 1). Soil pH reduced from 7.93 for the unamended control soil samples to 6.64 for amended soils at 30% DC application. The EC, however, enhanced from 1.43 mS cm⁻¹ (unamended control) to 2.87 mS cm⁻¹ (30% DC amended soils) (Table 1). This trend may be attributed to the lower pH and higher EC of the decanter cake used in the present study as compared with those of the unamended soil. Reduction in soil pH can also be attributed to the acidic nature of decanter cake and also to the release of humic acid as a result of decanter cake biodegradation (Singh and Agrawal 2010a, b, c). The heavy metal analysis of DC-amended soil revealed that there was no significant difference between the various treatments as the concentration of Cd, Pb, Cu, and Ni ranged from 0.08 to 0.88 mg g⁻¹, respectively (Table 1). Available P was found to be highest at 30% DC amendment (1.00 mg g⁻¹). The C/N ratio increased with increasing DC amendment ratios and was found to be highest at 30% DC ratio (18.72). Soil pH, moisture content, organic matter content, and a number of other factors influenced heavy metal availability in plants by regulating metal speciation, binding, precipitation reactions, and metal availability in soil solution. Singh and Agrawal (2010a, b, c) reported that as the pH decreases, there is a rise in the availability of heavy metals.

The biochemical response of lady's finger plants to various doses of decanter cake have been presented in (Table 2). Chlorophyll a (Chl a) decreased significantly with increasing treatment ratios (1.02 > 0.52 > 0.42 > 0.11 mg g⁻¹ fresh weight) for 0%, 10%, 20%, and 30% DC amendment, respectively, whereas chlorophyll b (Chl b) was reported to be highest at 30% DC treatment (0.45mg g⁻¹ fresh weight) (Table 2). Photosynthetic pigments are one of the targets of metal toxicity or any stress leading to damage of chloroplasts and inhibition in the production of chlorophyll. In the present study, the total chlorophyll content also reduced significantly with higher DC treatment. This conforms to the study of Singh and Agrawal (2007) that reported a decline in total chlorophyll content in *Beta vulgaris* (beet root) plants grown at 20% and 40% sewage sludge. Singh and Agrawal (2009) reported that total chlorophyll content decreased in lady's finger (*A. esculentus* L. var. Varsha uphar) by 10% at 20% sewage sludge amendment (SSA) and 47% at 40% SSA at 60 DAS as compared with those grown on unamended soil. On the contrary, total chlorophyll content in *Helianthus annus* (sunflower)

Table 1 Physicochemical properties of soil and decanter cake amendment ratios (Mean \pm 1SD)

Parameter	Unamended soil (0%)	10% DC	20% DC	30% DC
pH	7.93 ^a \pm 0.08	6.81 ^b \pm 0.06	6.71 ^b \pm 0.02	6.64 ^b \pm 0.02
EC (mS cm ⁻¹)	1.43 ^b \pm 0.02	1.67 ^b \pm 0.06	2.72 ^a \pm 0.03	2.87 ^a \pm 0.15
Available P (mg/g)	0.53 ^c \pm 0.03	0.72 ^b \pm 0.02	0.86 ^b \pm 0.06	1.00 ^a \pm 0.09
Cd (mg/g)	0.11 ^a \pm 0.00	0.11 ^a \pm 0.00	0.10 ^a \pm 0.01	0.08 ^a \pm 0.01
Pb (mg/g)	0.77 ^a \pm 0.11	0.67 ^b \pm 0.03	0.63 ^b \pm 0.04	0.73 ^a \pm 0.01
Cu (mg/g)	0.15 ^b \pm 0.02	0.15 ^b \pm 0.02	0.18 ^b \pm 0.04	0.30 ^a \pm 0.02
Ni (mg/g)	0.77 ^a \pm 0.01	0.73 ^a \pm 0.02	0.66 ^b \pm 0.03	0.75 ^a \pm 0.00
Organic carbon (%)	2.60 ^d \pm 0.03	10.2 ^c \pm 0.02	16.3 ^b \pm 0.01	21.8 ^a \pm 0.03
C:N ratio	10.74 ^c \pm 0.03	12.63 ^b \pm 0.01	12.72 ^b \pm 0.03	18.72 ^a \pm 0.02
Bulk density (gcm ⁻³)	1.00 ^a \pm 0.01	0.83 ^b \pm 0.03	0.87 ^b \pm 0.02	0.77 ^c \pm 0.01
Specific gravity	0.99 ^a \pm 0.13	0.84 ^b \pm 0.02	0.77 ^c \pm 0.01	0.74 ^c \pm 0.01

Different letters in each group show significant difference at $p < 0.05$.

plants in soil treated with organic compost application increased (El-Sabour et al. 1997). There was a significant difference ($p = 0.02$) in the Chl a/Chl b ratios of lady's finger plants as they decreased with elevated treatment rates. The reduction in Chl a/Chl b ratio suggests more damage to Chl a than to Chl b due to excess nutrients (Singh 2008). De Oliveira et al. (1994) reported that there were reductions in chlorophyll a and b in cotyledons of *Glycine max* cv Bessier and Doko as Cd concentrations increased. Vignesh et al. (2012) reported increase in protein, chlorophyll concentration, and antioxidant activity in spinach grown on vegetable waste amendments. The carotenoid content decreased significantly with increasing DC amendment ratios as compared with that of unamended control. Carotenoids are nonenzymatic antioxidants which protect the chlorophyll molecules against oxidative stresses (Halliwell 1987). Carotenoid and chlorophyll degradation is often observed in response to plant exposure to elevated heavy metals (Gallego et al. 1996; Luna et al. 1994; Singh and Agrawal 2007, 2009, 2010c). Foliar thiol content increased significantly at all DC amendment ratios. The formation of proteins is linked to thiol content as lower thiol means higher protein content. Phenol content increased with higher treatments as compared to that in the control. There was a

significant difference ($p = 0.04$) among the different treatments. Phenol content reflects the toxicity of metals (Singh 2008). Ascorbic acid content, a powerful antioxidant scavenging free oxy-radicals, increased significantly in lady's finger plants at all DC amendment ratios as compared to that of the unamended control. Ascorbic acid is an indicator of oxidative stress caused by excess nutrient contents (Singh 2008). Singh et al. (2004) reported an increase in ascorbic acid content of tomato plants grown in tannery sludge as compared to that in controls. Increase in ascorbic acid content is indicative of a defensive response against oxidative stress caused by increasing DC ratios.

Morphological analysis revealed that shoot lengths of the lady's finger plants decreased with increasing DC amendment ratios being highest at 30% DC amendment ratios (Table 3). Shoot length was found to reduce from 10% > 20% > 30% DC amendment ratios as compared to that of the control plant. No significant changes ($p = 0.42$) in root lengths had been observed due to decanter cake application as compared to unamended soil plants (Table 3). There was no significant difference ($p = 0.23$) in the leaf areas of the lady's finger plants grown among all the treatments; however, it was highest at 30% amendments (33 cm² plant⁻¹), whereas it was 22.8 cm² plant⁻¹ in

Table 2 Biochemical characteristics in lady's finger plants grown at different DC amendments (mean \pm 1SD) 25 DAS

Parameter	Unamended soil (0%)	10% DC	20% DC	30% DC
Ascorbic acid	0.967 ^d \pm 0.00	1.037 ^c \pm 0.03	1.047 ^b \pm 0.01	1.110 ^a \pm 0.01
Thiol	0.243 ^d \pm 0.15	8.513 ^c \pm 0.22	8.853 ^b \pm 0.28	9.117 ^a \pm 0.35
Phenol	10.103 ^d \pm 0.07	10.983 ^c \pm 0.27	13.197 ^a \pm 0.36	12.120 ^b \pm 0.52
Chlorophyll a	1.02 ^a \pm 0.04	0.520 ^b \pm 0.24	0.423 ^c \pm 0.08	0.113 ^d \pm 0.02
Chlorophyll b	0.347 ^c \pm 0.02	0.433 ^b \pm 0.19	0.29 ^d \pm 0.06	0.447 ^a \pm 0.07
Carotenoid	0.673 ^a \pm 0.05	0.357 ^c \pm 0.14	0.38 ^b \pm 0.02	0.120 ^d \pm 0.02
Total chlorophyll	1.370 ^a \pm 0.06	0.950 ^b \pm 0.10	0.71 ^c \pm 0.02	0.560 ^d \pm 0.05
Chl a/Chl b	2.960 ^a \pm 0.17	2.090 ^b \pm 1.01	1.79 ^c \pm 0.66	0.277 ^d \pm 0.10

Different letters in each group show significant differences at $p < 0.05$. Ratios were expressed in milligrams per gram.

Table 3 Selected growth characteristics of lady's finger (*Abelmoschus esculentus*) seedlings grown at different decanter cake amendment rates at 65 DAS (Mean±1SD)

Parameters	Control (0%)	10%	20%	30%
Shoot length (cm plant ⁻¹)	30.50 ^a ± 2.78	23.33 ^{ab} ± 3.06	21.50 ^b ± 5.27	17.5 ^b ± 0.50
Root length (cm plant ⁻¹)	10.77 ^a ± 0.75	11.4 ^a ± 1.44	14.12 ^a ± 11.12	6.1 ^a ± 0.66
Leaf area (cm ² plant ⁻¹)	22.8 ^a ± 13.32	28.67 ^a ± 1.2	30.67 ^a ± 10.26	33.0 ^a ± 20.29
Number of leaves (plant ⁻¹)	10.33 ^a ± 1.53	10.0 ^a ± 1.0	9.0 ^a ± 1.0	9.33 ^a ± 0.577
Leaf weight (g plant ⁻¹)	0.46 ^b ± 0.05	1.13 ^a ± 0.25	0.59 ^{ab} ± 0.11	0.72 ^{ab} ± 0.39
Shoot weight (g plant ⁻¹)	1.89 ^a ± 0.46	3.53 ^a ± 1.68	2.58 ^a ± 0.64	1.80 ^a ± 0.6
Root weight (g plant ⁻¹)	0.15 ^a ± 0.05	0.25 ^a ± 0.12	0.56 ^a ± 0.31	0.29 ^a ± 0.07
Fruit weight (g plant ⁻¹)	1.39 ^{ab} ± 0.10	2.80 ^a ± 0.61	1.64 ^{ab} ± 1.09	1.01 ^b ± 0.20
Total biomass (g plant ⁻¹)	2.62 ^b ± 0.12	4.49 ^a ± 0.30	2.49 ^b ± 1.03	1.64 ^b ± 0.27
Leaf Area Ratio (LAR) (cm ² g ⁻¹)	19.33 ^a ± 3.54	3.53 ^b ± 0.64	4.90 ^b ± 1.41	5.90 ^b ± 3.07
Leaf Weight Ratio (LWR) (gg ⁻¹)	0.28 ^a ± 0.01	0.27 ^a ± 0.03	0.30 ^a ± 0.02	0.36 ^a ± 0.08
Root Shoot Ratio (RSR) (gg ⁻¹)	0.34 ^b ± 0.03	0.34 ^b ± 0.01	1.63 ^a ± 0.06	1.12 ^a ± 0.03
Specific Leaf Area (SLA) (cm ² g ⁻¹)	118.50 ^a ± 34.83	65.31 ^a ± 3.61	50.77 ^a ± 7.37	46.81 ^a ± 27.77
Yield (g plant ⁻¹)	4.09 ^a ± 1.79	4.83 ^a ± 1.69	3.27 ^a ± 1.44	3.15 ^a ± 1.33

Different letters in each group show significant differences at $p < 0.05$.

lady's finger plants grown on 10% DC-amended soil. The number of leaves per plant also did not vary significantly due to decanter cake application compared to that of the control. The root length retardation observed at higher decanter cake application rate (30% DC) may be ascribed to high metal concentrations in the soil. Singh and Agrawal 2009) reported 20.6% and 21.5% decrease in root length, 16.6% and 9% decrease in shoot length, and 41.9% and 75% increase in leaf area of lady's finger plants grown at 20% and 40% sewage sludge amendment ratios as compared to those grown on unamended soil.

The yield of the lady's finger plants grown at 10% amendment was 4.83 g plant⁻¹, whereas for 20% and 30%, there were 31% (3.27 g plant⁻¹) and 34.8% (3.15 g plant⁻¹) reductions in yield as compared to the control (Table 3). For total biomass, shoot weight, and fruit weights, 10% amendments had highest values, at 4.49, 3.53, and 2.80 g plant⁻¹, respectively. Biomass accumulation is used for monitoring the effects of various environmental variables. LAR in 10% amendments (3.53 cm² g⁻¹) varied considerably from the control plant (19.33 cm² g⁻¹); however, it increased at higher concentrations of treatment. LWR for lady's finger plants was highest at 30% DC application (0.36g g⁻¹), and for RSR, the control and 10% varied significantly from 20% and 30% amendment. RSR denotes the portioning of photosynthates between above- and below ground parts. It may be possible that the availability of P through decanter cake may just be sufficient for the physiological need of the plants grown at 10% decanter cake, which showed higher biomass accumulation, and change in yield was insignificant as compared to the control. There was no significant difference ($p = 0.087$) in the

mean values of the SLA. The present study clearly showed that organic C, total N, available P, and nutrients contents in soil increased due to DC amendment. As the amendment ratios increased, the yields, biomasses, as well as morphological characteristics were negatively affected due to the high nutrient content of DC.

Conclusions

Decanter cake as a source of bio-fertilizer has great potential, but care should be taken as to the dosage applied. Results indicated that decanter cake amendments up to 10% may be a possible substitute for inorganic fertilizers with respect to lady's finger plants due to high nutrient content, yield and biomass, as well as plant growth. On the other hand, beyond 10% amendments, negative effects such as yellowing of leaves, stunted growth, and poor fruiting were observed. This is due to the excess of nutrients present. If decanter cake is used as recommended, it would, in the long run, reduce farmers' dependency on chemical fertilizers and associated health risks in oil-producing countries specifically in Malaysia.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AE is the main and corresponding author of this paper as it is part of her PhD research work. RPS was involved in the editing and the data analysis. MHI is the supervisor of the work and has given the approval for submission to this journal. All authors read and approved the final manuscript.

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Author details

¹Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia, Penang 11800, Malaysia. ²Institute of Environment and Sustainable Development, Banaras Hindu University, Varanasi 221005, India.

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